chromatography, high performance liquid chromatography (HPLC), differential centrifugation, filtration, gel filtration, membrane chromatography, affinity purification, biomolecular interaction analysis (BIA), ligand affinity purification, glutathione-S-transferase affinity chromatography, cellulose binding protein affinity chromatography, maltose binding protein affinity chromatography, avidin/streptavidin affinity chromatography, S-tag affinity chromatography, thioredoxin affinity chromatography, metal-chelate affinity chromatography, immobilized metal affinity chromatography, epitope-tag affinity chromatography, immunoaffinity chromatography, immunoaffinity capture, capture using bioreactive mass spectrometer probes, mass spectrometric immunoassay, and immunoprecipitation.

102. (Previously Presented) The method of claim 92 wherein the polypeptide masses are measured by a method selected from the group consisting of mass spectrometry, MALDI-TOF mass spectrometry, electrospray ionization mass spectrometry (ESI), tandem mass spectrometry (MS/MS), quadripole time of flight spectrometry (Q-TOF), Fourier transform ion cyclotron resonance (FTICR) mass spectrometry, gel electrophoresis, capillary electrophoresis, and high performance liquid chromatography (HPLC).

REMARKS

In an *Ex parte Quayle* action of March 29, 2005 that followed a conference between Examiner Marschel, Barbara Johnson (my counsel at the time) and I, claims 92 through 102 of the application were accepted in full.

However, in an Office Action dated 11-04-2005, the *Ex parte Quayle* action was rescinded in view of newly cited prior art of Blanc et al. (US Patent 5,891,695; filed September 25, 1993). Additional prior art of Blanc et al. (J. Bacteriology 177, 5206-5214, 1995) was cited in the Office Action of January 5, 2007 to which this amendment is responsive,

I have carefully read the Blanc et al. documents and I find nothing there that anticipates my invention. I believe that in rejecting my claims in view of Blanc et al., the examiner applied an incorrect and inappropriate definition of the term "reading frame" as used in my specification and claims.

As discussed in detail below, in my specification and claims the term "reading frame" refers to one of the six possible *overlapping translational reading frames* contained in a single polynucleotide molecule. This is the standard meaning of the term in the fields of genetics and molecular biology, and it is consistently used this way in the specification and claims, as demonstrated by specific references below, *and* it is used this way in previous communications of record to and from the USPTO and/or the WPTO, as demonstrated by specific references below.

In contrast, in the Blanc et al patent (5,891,695; filed September 25, 1993) and the Blanc et al. paper (J. Bacteriology 177, 5206-5214, 1995), the term "reading frame" means "open reading frame" - i.e., a *single* reading frame free of stop codons. Thus when Blanc et al refer to the presence of two reading frames within a single polynucleotide, they are referring to two long *non-overlapping open reading frames* - in particular two different natural genes within the polynucleotide. Indeed, in the later Blanc et al citation (the 1995 J. Bacteriology article), the term "reading frame" is never used except when preceded by the word "open", and the term is frequently abbreviated as "ORF".

The term "reading frame" appears more than 25 times in my specification and claims. In each case it refers to one of the six possible *overlapping translational reading frames* contained in a polynucleotide sequence. Specific examples showing this are given below

- Example 3 entitled "Identification of a Subcloned EcoRI Fragment of a Cloned Human Gene: Analysis of Peptides from Multiple Reading Frames [Paragraph 0043]. Here I describe the use of a commercially available vector (pTriplEx) that is designed to express polypeptides from more than one overlapping translational reading frame of a single polynucleotide. The predicted masses of peptides translated from *two different*

overlapping reading frames of 5 different polynucleotides (each a different EcoRI fragmet of a larger polynucleotide) are listed in tabular form [Paragraph 0046]. A reading of this table, and of the discussion provided in paragraph 0046, quoted below, makes it unambiguously clear that the peptides have been translated from *overlapping* reading frames of the polynucleotides of interest.

"[0046] To identify the nucleotide sequence adjacent to the pTriplEx' vector, each EcoRI site in the JO5584 sequence is identified and ligated, in silico, to the EcoRI site in the pTriplEx' vector. For each such in silico construct, *the amino acid sequences of the two expected hybrid translation products (from each of the start codons in the vector to the first in frame stop codons encountered in the insert) are calculated.* The mass of each peptide is calculated and all 10 peptide pairs are tabulated, as shown in the table below. Comparison of the experimental results (i.e., peptides of 4255 and 2635 Da.) with the values predicted in the table indicates that the insert begins at position 4028 of the reference sequence and proceeds in the forward direction. It is concluded that the 5' end of the sequence joined to the vector is GAATTCTCTTGGGTT TTGTGGTGTGCTAGACTTAATTACCCATGAATGATTT TGTCCTCTTCAGAAAATTTCAATAGCACATCTATT- AGTGTTTTTTAT . . . (1 st 100 nucleotides shown). The identification is confirmed by dideoxy sequencing from the plasmid using a primer 150 nucleotides 3' to the pTriplEx' EcoRI site".

- Example 7, [0055] Application of a Computer Program to Generate a Data Set of Mass Shifts for all Possible Single Nucleotide Substitutions in a Nucleotide Sequence. Here the input to the program includes "a choice by the user of which of the *six possible reading frames (3 forward and 3 reverse)*" [0056] is to be considered. The meaning of the term "reading frame" could not be more clear.

Yet another specific case is found in paragraph 0075 that discusses the analysis of mass data from peptides expressed from different overlapping reading frames:

• [0075] "Note also that the expression of polypeptides *from two reading frames* makes the analysis significantly more robust than if just one reading frame is used. For example, if just reading frame 1 is used, a shift of -14.03 Daltons could be due to an E-to-D substitution at amino acid 3, or to an E-to-D substitution at amino acid 4, or to an L-to-V substitution at amino acid 2. *When the additional reading frame data are considered*, however, each of these possibilities is distinguished from the others and the ambiguity is thereby eliminated. Indeed, *when up to six reading frames are considered*, there is little or no ambiguity for the great majority of substitutions, even for sequences as long as several hundred nucleotides".

Again, it is unambiguous that the multiple reading frames are *overlapping* sequences within the same polynucleotide.

• [0148] "For the purpose of expression, multiple promoters and translation start sites can be placed in the known sequence, on one or both sides thereof, so that the unknown sequence is *translated in up to six different reading frames*."

Again it is unambiguous that the different reading frames are different *overlapping* reading frames within the same polynucleotide.

The same meaning of the term "reading frame" is found in prior art cited by the WIPO in its International Search Report of 26 April 2000. In particular, the search report cites several pages from the 1986 textbook Molecular Cell Biology by James Darnell (Scientific American Books) in which the translation of polynucleotides in *multiple overlapping reading frames* is described. I have attached the cited prior art to this amendment. A reading of these pages (see for example Figure 4-7) makes it unambiguously clear that the reading frames that are presented overlap.

In summary then, it is unambiguous from the specification and claims of my application, and from the prior art that was of record in the prosecution of the application since 26

April, 2000, that the term "reading frame" as used in the application refers to one of the six possible overlapping reading frames of a polynucleotide.

Blanc et al., by contrast, use "reading frame" in an entirely different sense. For them "reading frame" is shorthand for "open reading frame" (ORF). It means an extended series of sense codons within a polynucleotide and nothing more. And when Blanc et al. refer to more than one ORF in a DNA molecule, they are referring to ORFs at different locations within the molecule, not at the same (overlapping) locations.

• This is illustrated by the following text found on pages 46 and 47 of Blanc et al's US patent 5,891,695.

"Analysis of the Nucleotide Sequences by Determination of the *Open Reading Frames*

"This example illustrates how it is possible to determine the *open reading frames* present in the nucleotide sequences defined in Example 7, and to identify the genes involved in the biosynthesis of pristinamycins I and pristinamycin II of S. pristinaespiralis SP92 as well as the polypeptides encoded by these genes.

"8.1. 5-kb BamHI-XhoI Fragment (pXL2045)

"This example illustrates how it is possible to determine *the open reading frames* present within the 5-kb BamHI-XhoI fragment isolated above and sequenced as described in Examples 6 and 7.

"We looked for the presence of *open reading frames* within the 5-kb BamHI-XhoI fragment utilizing the fact that Streptomyces DNA has a high percentage of G and C bases as well as a strong bias in the use of the codons of which the coding frames are composed (Bibb et al. 1984). The Staden and McLachlan (1982) method enables the probability of the coding frames to be calculated on the basis of the use of the codons of Streptomyces genes which are already sequenced and collated in a file containing 19673

codons obtained from the BISANCE data-processing server (Dessen et al. 1900).

"This method enabled *four highly probable open reading frames*, which are shown in the following table 10, to be characterized within the 5-kb BamHI-XhoI fragment. *They are designated frames 1 to 4* according to their position starting from the BamHI site. For each one, their length in bases, their position within the fragment (the BamHI site being located at position 1) and also the molecular weight in kDa of the corresponding protein are given. Frames 1, 3 and 4 are coded by the same strand and frame 2 by the complementary strand (FIG. 16).

"Frames 1 and 3 correspond respectively to the proteins SnaA (SEQ ID NO:17) and SnaB (SEQ ID NO:18) isolated above as described in Example 5 and for which the cloning of the genes is detailed in Example 6. In effect, the NH.sub.2 -terminal sequences of the products of ORFs 1 and 3 are identical to the NH.sub.2 -terminal sequences found for the proteins SnaA and SnaB, respectively, in Example 5.1.2, apart from the amino-terminal methionine which has been excized. Moreover, the molecular masses calculated from the sequences are comparable to the apparent molecular masses of the proteins SnaA and SnaB, estimated, respectively, in SDS-PAGE as described in Example 5.

Comparison of the product of *open reading frame no. 4* with the protein sequences contained in the NBRF bank reveals a homology with various S-adenosylmethionine (or SAM) synthases, in particular of E. coli (Markham et al., (1984), of rat (Horikawa et al., 1989) and of S. cerevisiae (Thomas et al., 1988). The percentage homology values calculated over the whole of the sequence using Kanehisa's (1984) algorithm vary from 51.8 to 55.4%.

"These sequence comparisons hence enable it to be demonstrated that the product of *open reading frame no. 4* is an SAM synthase involved in the biosynthesis of pristinamycins I or II. This gene was designated samS (SEQ ID no. 4).

"The demonstration of the involvement of the samS gene in the biosynthesis of pristinamycins is confirmed by the construction of the SP92 mutant disrupted in this gene, as described in Example 9.2.

"Comparison of the sequence of the product of *open reading frame no. 2* with the protein sequences contained in the Genpro bank reveals that an internal portion of this protein is 36% homologous with an internal portion of the first open reading frame of the insertion sequence (IS891) of Anabaena (Bancroft and Wolk, 1989). This result suggests that *open reading frame no. 2*, designated ORF 401, belongs to an insertion sequence, and that there is hence an insertion sequence located between the snaA and snaB genes."

The fact that the ORFs *do not overlap* is made particularly clear in Figure 32 of the Blanc patent that is copied below. Figure 2 (page 5209) in the Blanc et at paper (J. Bacteriology 177, 5206-5214, cited in the January 5 2007 Office Action) shows the same thing - that *the open reading frames do not overlap.*

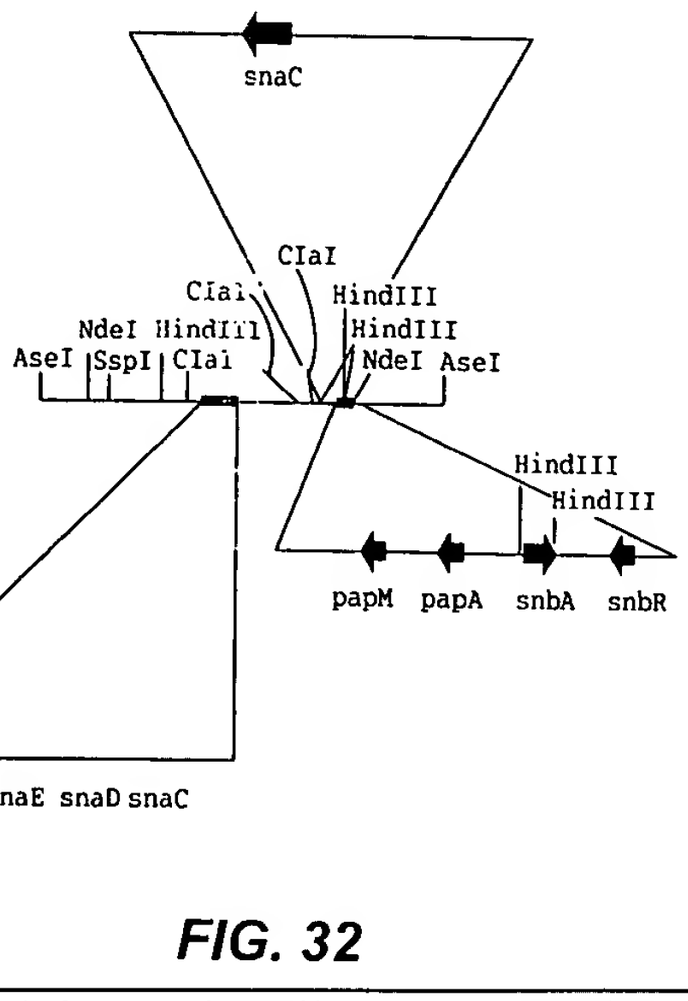


FIG. 32

In summary then, Blanc et al do not describe or contemplate the expression of multiple polypeptides from overlapping reading frames of a polynucleotide having homology to a defined DNA sequence; rather they consider individual polypeptides that might be naturally expressed from individual *non-overlapping* ORFs of a DNA molecule. Consequently their teachings do not anticipate my invention.

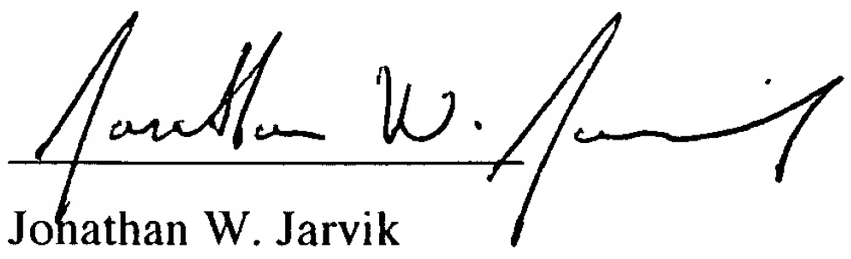
My invention has been described and applied in four peer-reviewed publications of which I am a co-author, each of which is attached to this amendment. At no point in the review process for these papers did any reviewer or editor express confusion about the meaning of the term reading frame. Nor did any reviewer or editor suggest that the multiple reading-frame process was anticipated by Blanc et al. or by any other prior art. Indeed, if the expert peer reviewers of the papers knew that the invention previously existed, they would have required that the prior art be cited in those publications.

Finally, the examiner cites the Jermutus et al. review on the subject of cell-free translation as prior art in the January 5, 2007 Office Action. However, while live-cell or cell-free translation is part of the claimed process, there is no independent claim directed just to translation, nor does the specification suggest that live-cell or cell-free translation is novel or inventive in and of itself. Since Jermutus et al. does not suggest or anticipate the process of any of claims 92-102, it provides no basis for rejection of the claims.

CONCLUSION

In view of the facts and analyses presented above, I submit that the prior art cited by the examiner does not anticipate any of the claims provided herein, and I respectfully request withdrawal of the rejections and allowance of claims 92-102.

Respectfully submitted,



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